Online Supplementary Material

Supplementary Materials and Methods

Oligonucleotide Primer Sequences:

Rb(K810W) mutant

Forward 5'-GAACATCTATATTTCACCCCTGTGGAGTCCATATAAAATTTCAGAAGGTC-3'

Reverse 5'- GACCTTCTGAAATTTTATATGGACTCCACAGGGGTGAAATATAGATGTTC-3'

ChIP Assay

Promoter	Forward	Reverse
casp-7	5'-TTTGGGTCACTTGGAGCGCG-3'	5'-AAGAGCCCAAAGCGACCCGT-3'
cyclin A	5'-TACTTGAACTGCAAGAACAGCCGCGCTC-3'	5'-GATCAGCCTGCGGCGCCAAGCAGCGT-3'
TK	5'-TCCCGGATTCCTCCCACGAG-3'	5'-TGCGCCTCCGGGAAGTTCAC-3'
p21	5'-GGGTGTAGGGAGATTGGTTCAATG-3'	5'-CCGCTGACCCACTCTGGCAGGC-3'
p73	5'-CTCTGCCGAAGATCGCGGTCGG-3'	5'-GGCCGCGTCCAAGTCGGGGTCC-3'
CDC2	5'- GCTTGCGCTCGCACTCAGTTGGCG-3'	5'- AGTGCGAGCAGTTTCAAACTCAC-3'
CDC25A	5'-TCTGCTGGGAGTTTTCATTGACCTC-3'	5'- TTGGCGCCAAACGGAATCCACCCAATC-3'
β-actin	5'-ACGCCAAAACTCTCCCTCCTCCTC-3'	5'-CATAAAAGGCAACTTTCGGAACGGC-3'

Assay of Calpain Activity

For assay of calpain activity, Calpain-1 (50 nM) in the presence or absence of calpastatin (from 10-500 nM) or SerpinB2 (from 10-500 nM) were incubated with 100 μM fluorogenic peptide substrate (Suc-Leu-Tyr-AMC)(Calbiochem) in 25 mM Hepes pH 7.5, 100 mM NaCl, 0.5 mM DTT and 4 mM $CaCl_2$ at 35°C over a 20-min period. Changes in fluorescence at 460 nm were monitored with time on a PerkinElmer Life Sciences HTS 7000 BioAssay Reader. Assays were performed in triplicate. Initial rates were measured for each inhibitor concentration.

Supplementary Figure 1

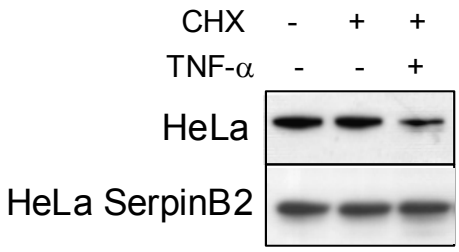

Figure S1. SerpinB2 protects HeLa cells from the loss of Rb during initiation of TNFα induced apoptosis. HeLa and HeLa expressing SerpinB2 (S1a) (18) cells were treated with CHX alone or in combination with 1 ng/ml of TNFα for 3 hrs. Cell lysates were immunoblotted for Rb using Rb(G3-245) and exposed to detect Rb signals (the HeLa blot is a 10x longer exposure than HeLa SerpinB2). Signals were analyzed by densitometry and values normalized to GAPDH. Densitometric analysis of the blots reveal a 45% decrease in full length Rb in TNF/CHX treated HeLa cells relative to HeLa cells treated with CHX alone, whereas Rb is stabilized in the presence of SerpinB2.

Supplementary Figure 2

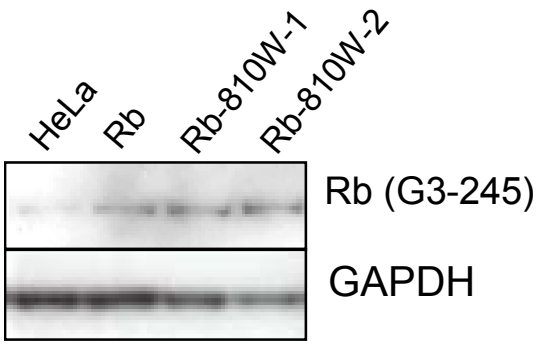

Figure S2. HeLa cells were transfected with plasmids encoding wild type Rb (Rb) or the Rb-810W calpain cleavage site mutant, and stable clones isolated. Lysates of untransfected HeLa cells, one wild type Rb clone and two Rb-810W clones were immunoblotted for Rb and GAPDH. A higher level of both wild type Rb and the Rb-810W mutant is detected compared to untransfected HeLa cells.